

University of Dundee

Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of Sarcopenia in older adults, the PROVIDE study

Bauer, Jürgen M.; Verlaan, Sjors; Bautmans, Ivan; Brandt, Kirsten; Donini, Lorenzo M.; Maggio, Marcello

Published in:

Journal of the American Medical Directors Association

DOI:

[10.1016/j.jamda.2015.05.021](https://doi.org/10.1016/j.jamda.2015.05.021)

Publication date:

2015

Licence:

CC BY-NC-ND

Document Version

Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Bauer, J. M., Verlaan, S., Bautmans, I., Brandt, K., Donini, L. M., Maggio, M., McMurdo, M. E. T., Mets, T., Seal, C., Wijers, S. L., Ceda, G. P., De Vito, G., Donders, G., Drey, M., Greig, C., Holmbäck, U., Narici, M., McPhee, J., Poggiogalle, E., ... Cederholm, T. (2015). Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of Sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. *Journal of the American Medical Directors Association*, 16(9), 740-747. <https://doi.org/10.1016/j.jamda.2015.05.021>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



JAMDA

journal homepage: www.jamda.com

Original Study

Effects of a Vitamin D and Leucine-Enriched Whey Protein Nutritional Supplement on Measures of Sarcopenia in Older Adults, the PROVIDE Study: A Randomized, Double-Blind, Placebo-Controlled Trial



Jürgen M. Bauer MD, PhD ^{a,*}, Sjors Verlaan MSc ^{b,c}, Ivan Bautmans PhD ^d, Kirsten Brandt PhD ^e, Lorenzo M. Donini MD, PhD ^f, Marcello Maggio MD, PhD ^g, Marion E.T. McMurdo MD, PhD ^h, Tony Mets MD, PhD ^d, Chris Seal PhD ^e, Sander L. Wijers PhD ^b, Gian Paolo Ceda MD ^g, Giuseppe De Vito MD, PhD ⁱ, Gilbert Donders MD, PhD ^j, Michael Drey MD ^k, Carolyn Greig PhD ^l, Ulf Holmbäck PhD ^m, Marco Narici PhD ⁿ, Jamie McPhee PhD ^o, Eleonora Poggiogalle MD ^f, Dermot Power MD, PhD ^p, Aldo Scafoglieri PhD ^d, Ralf Schultz MD, PhD ^q, Cornel C. Sieber MD ^r, Tommy Cederholm MD, PhD ^m

^a Department of Geriatric Medicine, Carl von Ossietzky University, Oldenburg, Germany

^b Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands

^c Department of Internal Medicine, Section of Gerontology and Geriatrics, VU University Medical Center, Amsterdam, The Netherlands

^d Frailty in Ageing research group (FRIA), Vrije Universiteit Brussel (VUB), Brussels, Belgium

^e Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, Newcastle University Institute for Ageing, Newcastle University, Agriculture Building, Kings Road, Newcastle upon Tyne, United Kingdom

^f Department of Experimental Medicine, Section of Medical Pathophysiology, Endocrinology and Human Nutrition, "Sapienza" University of Rome, Rome, Italy

^g Department of Clinical and Experimental Medicine, Section of Geriatrics, Movement Disorders and Prevention of Disability Unit Food Sciences Unit and Endocrinology of Aging Unit, University of Parma, University Hospital, Parma, Italy

^h Ageing and Health, Ninewells Hospital and Medical School, University of Dundee, Ninewells Hospital, Dundee, United Kingdom

ⁱ Institute for Sport and Health, University College Dublin, Belfield, Dublin, Ireland

^j Femicare, Clinical Research for Women, Tienen, Belgium

^k Medizinische Klinik und Poliklinik IV, Schwerpunkt Akutgeriatrie, Klinikum der Universität München (LMU), Munich, Germany

^l School of Sport, Exercise and Rehabilitation Sciences and Centre for Musculoskeletal Ageing Research, University of Birmingham, Edgbaston, Birmingham, United Kingdom

^m Department of Public Health and Caring Sciences/Clinical Nutrition and Metabolism, Department of Geriatric Medicine, Uppsala University Hospital, Uppsala, Sweden

ⁿ Faculty of Medicine, MRC ARUK Centre for Musculoskeletal Ageing Research, Royal Derby Hospital, University of Nottingham, Derby, United Kingdom

^o School of Healthcare Science, Manchester Metropolitan University, All Saints, Manchester, United Kingdom

^p Department of Medicine for Older Persons, Mater Misericordiae University Hospital and University College Dublin, Belfield, Dublin, Ireland

^q St-Marien Hospital Clinic for Geriatrics, Cologne, Germany

^r Institute for Biomedicine on Ageing, Friedrich-Alexander-University Erlangen-Nürnberg, Nürnberg, Germany

A B S T R A C T

Keywords:

Sarcopenia
nutritional supplementation
muscle mass
lower extremity function
protein

Background: Age-related losses of muscle mass, strength, and function (sarcopenia) pose significant threats to physical performance, independence, and quality of life. Nutritional supplementation could positively influence aspects of sarcopenia and thereby prevent mobility disability.

Objective: To test the hypothesis that a specific oral nutritional supplement can result in improvements in measures of sarcopenia.

The authors declare no conflicts of interest.

This study was financially supported and study products were provided by Nutricia Research, Nutricia Advanced Medical Nutrition. The funding source was involved at all stages of the study. The final interpretation of the study results, review, and decision to submit the manuscript was performed by independent researchers with no affiliation to the funding source.

J.M.B. and S.V. contributed equally to this study, as did C.C.S. and T.C.

* Address correspondence to Jürgen M. Bauer, MD, PhD, Department of Geriatric Medicine, Carl von Ossietzky University, Oldenburg, Rahel-Straus-Strasse 10, 26133 Oldenburg, Germany.

E-mail address: bauer.juergen@klinikum-oldenburg.de (J.M. Bauer).

<http://dx.doi.org/10.1016/j.jamda.2015.05.021>

1525-8610/© 2015 AMDA – The Society for Post-Acute and Long-Term Care Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Design: A multicenter, randomized, controlled, double-blind, 2 parallel-group trial among 380 sarcopenic primarily independent-living older adults with Short Physical Performance Battery (SPPB; 0–12) scores between 4 and 9, and a low skeletal muscle mass index. The active group ($n = 184$) received a vitamin D and leucine-enriched whey protein nutritional supplement to consume twice daily for 13 weeks. The control group ($n = 196$) received an iso-caloric control product to consume twice daily for 13 weeks. Primary outcomes of handgrip strength and SPPB score, and secondary outcomes of chair-stand test, gait speed, balance score, and appendicular muscle mass (by DXA) were measured at baseline, week 7, and week 13 of the intervention.

Results: Handgrip strength and SPPB improved in both groups without significant between-group differences. The active group improved more in the chair-stand test compared with the control group, between-group effect (95% confidence interval): -1.01 seconds (-1.77 to -0.19), $P = .018$. The active group gained more appendicular muscle mass than the control group, between-group effect: 0.17 kg (0.004 – 0.338), $P = .045$.

Conclusions: This 13-week intervention of a vitamin D and leucine-enriched whey protein oral nutritional supplement resulted in improvements in muscle mass and lower-extremity function among sarcopenic older adults. This study shows proof-of-principle that specific nutritional supplementation alone might benefit geriatric patients, especially relevant for those who are unable to exercise. These results warrant further investigations into the role of a specific nutritional supplement as part of a multimodal approach to prevent adverse outcomes among older adults at risk for disability.

© 2015 AMDA – The Society for Post-Acute and Long-Term Care Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Preserving physical mobility, function, and ultimately independent living is of utmost importance for frail older adults.¹ Sarcopenia, the age-related loss of muscle mass, strength, and function^{2,3} makes up a large component of physical frailty.⁴ It is a strong risk factor for reduced mobility, events like falls and fractures,⁵ and is directly related to rates of hospital and long-term care admissions,⁶ increased disability,⁷ reduced independence, quality of life, and ultimately resulting in death.⁸ The onset and progression of sarcopenia is multidimensional involving physical inactivity, altered metabolism, neuromuscular deterioration, and marginal nutrient intakes and absorption.² The component of marginal nutrient intakes is of foremost interest here, because it is a modifiable risk factor of sarcopenia. Particularly protein, essential amino acids, leucine, and vitamin D intake are identified as important factors in the management of sarcopenia.⁹ Inadequate protein intake [ie, below the recommended dietary allowance of 0.8 g/kg body weight (BW)/day] as well as vitamin D status (ie, 25-hydroxyvitamin D < 50 nmol/L) are often cited as being strongly correlated with lower muscle mass,^{10,11} physical performance and muscle strength,^{12,13} and a risk for falls and fractures.¹⁴ Even in the presence of total per-day adequate protein intake, older adults' muscle is less sensitive to anabolic stimuli,¹⁵ such as resistance exercise¹⁶ and mixed meals,^{17,18} compared with younger adults, a condition known as "anabolic resistance."¹⁹

Recent recommendations focus on daily protein intakes that should be at least 1.0 to 1.2 g/kg BW/day for healthy older people, and 1.2 to 1.5 g/kg BW/day for geriatric patients with acute and chronic diseases.^{17,20} Further, given the blunted sensitivity of older muscles to low doses of amino acids, there are indications that dietary protein should be appropriately distributed to at least 25 to 30 g of high-quality protein per meal containing approximately 2.5 to 2.8 g of leucine, to stimulate muscle protein synthesis.¹⁷ These concepts of intake timing, as well as protein quality, are subjects of several recent studies.^{21,22} In a recent study, a bolus intake of a leucine-enriched, whey protein nutritional supplement stimulated acute postprandial muscle protein synthesis in both healthy and sarcopenic elderly.²³

Therefore, we hypothesized that providing a targeted nutritional supplement containing whey protein, enriched with leucine and vitamin D in a timely bolus amount, would result in the accretion of muscle protein and improvements of muscle strength and function independent of physical exercise among nonmalnourished sarcopenic older adults at high risk for disability. We explored the efficacy and safety of this concept compared with an iso-caloric control supplement for improving measures of sarcopenia: lower-extremity

function (Short Physical Performance Battery [SPPB] and its individual components), muscle strength (handgrip strength), and muscle mass [appendicular muscle mass by dual X-ray absorptiometry (DXA)].

Methods

Design and Participants

This was a 13-week, multicenter, randomized, controlled, double-blind, 2 parallel-group study among non-protein-energy malnourished older participants with mobility limitations. The study protocol was approved by institutional review boards at each location and registered under the Dutch trials register with the identifier: NTR2329 (<http://www.trialregister.nl/trialreg>). Study procedures were performed in accordance with the Declaration of Helsinki ethical principles for medical research involving human subjects.

Participants were recruited from 18 study centers in 6 European countries: Belgium, Germany, Ireland, Italy, Sweden, and the United Kingdom. Older adults (≥ 65 years) were screened for mild to moderate limitations in physical function (SPPB score 4–9), and for low skeletal muscle mass index [SMI; (skeletal muscle mass/BW * 100) $\leq 37\%$ in men and $\leq 28\%$ in women] using bioelectric impedance analysis (BIA 101; Akern, Florence, Italy)²⁴ because of its feasibility for an extensive screening process at multiple research sites. Further, participants were then eligible to participate if they had a body mass index (BMI) between 20 and 30 kg/m², no major cognitive impairment (Mini Mental State Examination score ≥ 25), and were able and willing to provide informed consent. Potential participants were excluded if they had comorbidities such as kidney or liver failure, malignancies over the past 5 years, anemia, or acute inflammation (C-reactive protein concentration > 10 mg/L), or presented with contraindications for calcium/vitamin D supplementation and/or were using medication interfering with the nutritional intervention.

Intervention

Participants were randomized to receive either the active or an iso-caloric control product. The active product contained, per serving, 20 g whey protein, 3 g total leucine, 9 g carbohydrates, 3 g fat, 800 IU vitamin D, and a mixture of vitamins, minerals, and fibers, whereas the iso-caloric control product did not contain any protein or micronutrients, and only carbohydrates, fat, and some trace elements

(Supplemental Table 1). Both were delivered as 40 g powder to be reconstituted with 100 to 150 mL water and consumed twice daily before breakfast and lunch to provide an adequate bolus of protein in addition to the meals.

Stratification and Randomization

Permuted block randomization (block size 4) to the active or control group was stratified for SPPB categories 4 to 6 and 7 to 9 and study center. The randomization sequence was computer-generated by a blinded statistician not involved in data collection or analysis. All investigators, study staff, and participants were blinded to group allocations, and the randomization code was not broken until statistical modeling of the primary and secondary outcomes was complete.

Outcome Measures

Blinded research staff assessed the outcomes during designated visits at week 7 and 13.

One of the 2 primary outcome measures, handgrip strength, was measured using a hydraulic hand dynamometer (Jamar; Preston, Jackson, MO). Two consecutive measures of grip strength in both hands were recorded to the nearest kilogram with the participant in an upright position and the arm of the measured hand parallel to the body. Maximum grip strength was calculated by taking the average of the highest measurement from both hands.

The other primary outcome measure, SPPB, consisted of the 3 components: gait speed (4-meter walk at a usual pace), chair stand test (time required to rise 5 consecutive times from a chair without arm rests), and balance (3 different standing balance tests) according to the method outlined in Guralnik et al.⁶ Each component was scored from 0 (not possible) to 4 (best performance) and summed in a total score ranging from 0 to 12. The individual outcomes related to physical function: chair rise test, gait speed, and balance score, were predefined as separate secondary outcomes.

Other secondary outcomes were appendicular muscle mass (by DXA) and questionnaires of self-reported physical activity, activities of daily living, and health-related quality of life. DXA (different models from Hologic, Bedford, MA, and Lunar, Fairfield, CT) was used to measure appendicular muscle mass at baseline and week 13. Central blinded analysis of raw DXA data from all sites was performed at Vrije Universiteit Brussel, to ensure uniformity in the analysis.

Self-reported amount of physical activity was measured using the European version of the Physical Activity Scale for the Elderly (PASE).²⁵ The Barthel index²⁶ measured the level of independence in activities of daily living with possible scores between 0 and 100 (highest scores best). Health-related quality of life was measured using the EQ-5D,²⁷ both as an index and as a visual analogue scale (VAS) between 0 and 100.

Product compliance was measured using self-completed intake diaries. Adequate compliance was defined as having consumed 10 of the possible 14 servings per week. Dietary assessment was done at baseline and week 13 using 3-day prospective diet records for 2 week-days and 1 weekend day. Additional energy and protein intakes from both supplements were added to the habitual 3-day intakes (ie, nonsupplementary intake) to assess total intakes.

Fasting glucose and insulin were measured at screening, serum 25-hydroxy-vitamin D and insulinlike growth factor 1 (IGF-1) at baseline, week 7, and week 13. Safety assessments included the examination of participant medical history, recording of medication use, nutritional supplements, and adverse events via telephone calls throughout the intervention and at each of the visits. Additional safety assessments were done at the designated visits. These included

monitoring vital signs, gastrointestinal tolerance, evaluating laboratory parameters related to liver and renal function, and inflammatory status.

Statistical Analyses

This study was powered to detect an effect size of 1.9 kg for handgrip strength^{28,29} and a 0.5-point difference in SPPB.³⁰ Assuming an α -value of 0.025, a 2-sided effect, and using the Hochberg principle for 2 primary outcomes, a sample size of 300 gave 80% power to observe an effect. Eighty additional participants were randomized under the guidance of the data monitoring committee following the blinded interim analysis.

Analyses were performed as intention-to-treat, defined as all participants randomized, regardless of whether they finished the full study protocol. Baseline unadjusted means and SDs and week 7 and week 13 unadjusted mean changes from baseline [medians and interquartile ranges (IQRs) for non-normal data] are presented. A mixed model for repeated measures (MMRM) was performed including the baseline value in the outcome vector and fixed factors for treatment and time (continuous). In this model, the treatment by time interaction coefficient estimates the potentially differential change in outcomes over time between active and control group. No adjustments were made for multiple testing for secondary outcomes due to the exploratory nature of the study. Continuous variables that were positively skewed were log-transformed before analysis in the MMRM. The MMRM for all outcomes included the predefined covariates baseline protein intake, age, and sex. For 16 participants, imputation was performed for missing baseline protein intake using the overall group mean intake. Missing values in outcome variables were not imputed because mixed models can handle missing data by maximum likelihood.³¹ The Mann-Whitney *U* test was used for categorical variables that could not be used in the MMRM model.

All statistical analyses were done using SAS software (version 9.4; SAS, Inc, Cary, NC) according to the predefined statistical analysis plan. The statistical analyses were repeated by independent statisticians (Julius Centre, Utrecht University), who confirmed the findings.

Results

Between June 30, 2010, and May 30, 2013, 1240 older adults were screened for participation, 380 of whom were randomized to the intervention or control groups (Figure 1). After the 13-week intervention, 302 participants completed all 3 study visits (79% completion rate). Baseline background characteristics were similar in both groups (Table 1). The mean age of the population at enrolment was 77.7 years, most of whom were women (65%), and living independently (87%). All participants had low muscle mass, a mean SPPB score of 7.5 (Table 2), a mean BMI of 26.1 kg/m², and were non-malnourished based on the Mini Nutritional Assessment Short-Form (99.5%). Intervention compliance was high (median: 93%) from baseline to follow-up, and did not differ between groups.

There was no significant difference in handgrip strength changes over time between the control and active groups. Handgrip strength improved significantly over time in the intervention group ($P = .005$), whereas there was likely no time effect in the control group ($P = .06$). SPPB scores increased significantly over time in both active and control groups ($P < .001$), but with no significant treatment \times time effect (Table 2).

Chair-stand time improved significantly in both groups over time ($P < .001$), with a significantly greater improvement in the active group compared with control ($P = .018$). Both groups improved significantly over time in gait speed ($P < .001$), but the

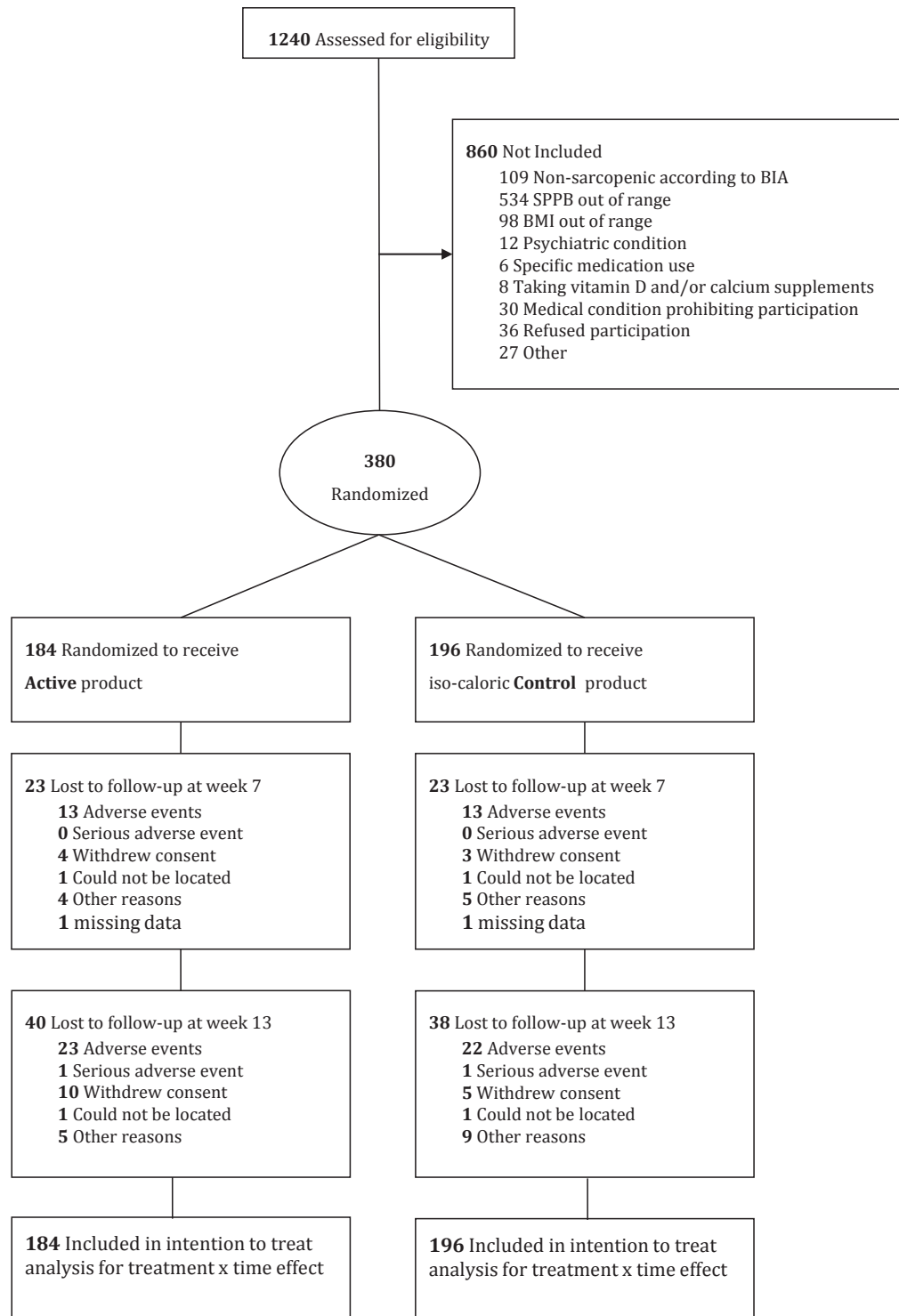


Fig. 1. Participant screening, randomization and follow-up.

treatment \times time effect was not significant. Balance scores remained unchanged both over time and by treatment (Table 2).

The increase in appendicular muscle mass was significantly greater in the active group than the control group, leading to a mean estimated difference of 0.17 kg (95% confidence interval [CI] 0.004–0.338) ($P = .045$) (Figure 2). There was a significant gain over time in appendicular muscle mass in the active group alone ($P < .001$).

No treatment \times time effects were observed in the PASE questionnaire, Barthel index, or quality of life as measured with the EQ-5D index. There was a significant time effect observed in the active group in the quality of life EQ-5D VAS score, leading to a trend for a mean treatment \times time effect of 2.5 mm (95% CI -0.17 – 5.16 ; $P = .07$).

Habitual dietary energy intakes, without supplements, decreased significantly over time in both groups, whereas supplementation in

Table 1
Baseline Demographic and Clinical Characteristics

	Active n = 184	Control n = 196
Age, mean (SD), y	77.3 (6.7)	78.1 (7.0)
Sex, female n (%)	120 (65.2)	129 (65.8)
Living situation, n (%)		
Institutionalized	18 (9.8)	19 (9.7)
Home care	4 (2.2)	10 (5.1)
Living independently	162 (88.0)	167 (85.2)
Mini Mental State Examination, median (IQR)	29.0 (27.0–30.0)	29.0 (28.0–30.0)
Hemoglobin concentration, median (IQR), mmol/L	8.4 (7.9–8.9)	8.5 (8.0–8.9)
BMI, mean (SD), kg/m ²	26.0 (2.5)	26.2 (2.8)
Mini Nutritional Assessment Short-Form (MNA-SF), n (%)		
Malnutrition	1 (0.5)	1 (0.5)
Risk of malnutrition	15 (8.2)	19 (9.7)
No malnutrition	168 (91.3)	176 (89.8)
Protein intake, median (IQR), g/kg body weight/day	1.0 (0.9–1.2)	1.0 (0.8–1.2)
Fasting glucose concentration, median (IQR), mmol/L	5.2 (4.9–5.8)	5.2 (4.9–5.7)
Fasting insulin concentration, median (IQR), mU/L	9.0 (5.0–13.0)	9.0 (6.0–14.0)
Handgrip strength male, median (IQR), kg	26.8 (22.0–30.8)	27.1 (22.0–32.1)
<30 kg, n (%)	45 (70.3)	45 (69.2)
Handgrip strength female, median (IQR), kg	16.5 (13.5–21.5)	16.8 (14.2–20.5)
<20 kg, n (%)	80 (69.6)	94 (74.0)
Gait speed n (%), <0.8 m/s	101 (54.9)	109 (55.6)
SMI (BIA), n (%)		
Normal SMI	0 (0)	0 (0)
Class I sarcopenia*	154 (84)	164 (84)
Class II sarcopenia†	30 (16)	32 (16)
Appendicular muscle mass (DXA), mean (SD), kg	17.9 (4.1)	17.5 (3.8)

BIA, bioelectric impedance analysis; SMI, skeletal muscle mass index; DXA, dual energy x-ray absorptiometry.

*Class I sarcopenia (skeletal muscle mass, kg/body mass, kg * 100), men: 31%–37%, women: 22%–28%.

†Class II sarcopenia (skeletal muscle mass, kg/body mass, kg * 100), men: <31%, women: <22%.

both groups led to a significant increase in total energy intakes at week 13 ($P < .001$).

Habitual protein intakes were not different between groups at baseline and did not change significantly from baseline to week 13, nor did it differ between groups. Participants in the active group alone showed an increased total protein intake at week 13 ($P < .001$) (Table 3).

At baseline, 25-hydroxyvitamin D concentrations were 50 nmol/L or lower in 51% and 53% of the participants in the control and active group, respectively. In the active group, 25-hydroxyvitamin D concentrations improved significantly ($P < .001$) at week 13 (Table 3). At follow-up, 1 participant in the control group (<1%) and 4 participants in the active group (3%) showed 25-hydroxyvitamin D concentrations of 125 nmol/L or higher. There was a significant time × treatment effect in IGF-1, with an effect size of: 12.6 µg/L (95% CI 7.4–18.1; $P < .001$).

Based on (serious) adverse events, gastrointestinal tolerance, and laboratory parameters, both study products seem safe and the data do not give rise to any concern. There were no statistically significant differences in incidence of (serious) adverse events between the groups (Figure 1).

Discussion

This 13-week specific nutrition intervention among non–protein-energy malnourished older adults with mobility limitations did not

lead to significant differences in SPPB or handgrip strength. There were, however, significant gains in muscle mass and improvements in chair stand ability in the active group versus control. This suggests that a vitamin D and leucine-enriched whey protein nutritional supplement, which stimulated muscle protein synthesis in an acute setting,^{32,33} could improve measures of sarcopenia over a 3-month intervention.

Exercise should be considered as the standard treatment for increasing muscle strength and improving physical performance among adults with sarcopenia.^{34–36} In this robust trial, however, we aimed to investigate the isolated effect of a targeted nutritional intervention. This area has mostly focused on the acute effects on muscle protein synthesis. Very few longer-term nutritional intervention studies have been performed to assess changes in muscle mass, strength, and function among older adults at risk for mobility disability.³⁷ Fiatarone and colleagues³⁸ did not show any effect of a nutritional supplement alone given for 10 weeks to 100 frail nursing home residents. In a more recent study in prefrail and frail older adults ($n = 65$), Tieland and colleagues³⁹ demonstrated that nutritional supplementation led to improvements in SPPB, but did not result in differences in muscle mass or strength.

In this study, we selected sarcopenic older adults characterized by both low muscle mass and function, using a nutritional supplement specifically targeted for aging muscle. To our knowledge, this is the only study using nutritional supplementation alone to result in an increased muscle mass among this population. Adults older than 70 years lose on average 5% to 10% of their muscle mass per decade.^{40–42} The approximate gain of 1% total appendicular muscle mass that we observed after 13 weeks of intervention would translate, therefore, into saving 1 to 2 years of muscle mass decline.

High habitual protein intake could be correlated with appendicular muscle mass retention.¹⁰ At baseline, habitual protein intakes in both groups were above the recommended dietary allowance of 0.8 g/kg per day for adults.⁴³ The active group alone achieved a higher total protein intake of 1.5 g/kg per day, which is in line with recent PROT-AGE and European Society for Clinical Nutrition and Metabolism recommendations for geriatric patients (1.2–1.5 g/kg per day).^{17,20} Beyond protein quantity, quality and timing of the protein supplementation are also considered crucial determinants for retention of muscle mass and function.^{15,17,20} In short-term studies, bolus intake of whey protein and leucine provided sufficient levels of essential amino acids, particularly leucine, required to elicit an appropriate acute muscle protein synthesis response.^{32,33,44} The leucine-enriched whey protein blend seems to be an appropriate approach to preserve muscle mass and function in older sarcopenic adults, possibly through the timely stimulation of muscle protein synthesis and the anabolic environment, as suggested by the IGF-1 increase we observed.⁴⁵

Additionally, serum 25-hydroxyvitamin D concentrations between 60 and 75 nmol/L are suggested to be optimal for lower-extremity strength, and falls and fracture prevention.⁴⁶ During the 3-month supplementation of at least 800 IU daily vitamin D in the active group, baseline serum 25-hydroxyvitamin D concentrations of median 48 (IQR 34–66) nmol/L (<50 nmol/L is considered insufficient) increased by median 25 (IQR 14–39) nmol/L, which resulted in concentrations within the optimal range.⁴⁶ Reversing the vitamin D inadequacies, in combination with leucine and amino acids, could have contributed to the favorable effect we observed on muscle parameters.⁴⁷

This study is not without limitations. Our primary outcome measurement, handgrip strength, is a well-validated proxy measurement for lower-body strength,⁴⁸ but is less sensitive to intervention changes than other measures of strength. A study showed that although handgrip and leg-press strength are well-correlated with each other and with muscle mass, leg strength showed an intervention effect, whereas handgrip strength did not.⁴⁹

Table 2
Muscle Strength and Function Outcomes

	Mean (SD)	Change From Baseline, Mean (SD)		Estimated Between-Group Difference Mean (95% CI) Active – Control	<i>P</i> *
	Baseline	Week 7	Week 13		
Handgrip strength, kg					
Active [†]	20.9 (7.9)	0.20 (3.2)	0.79 (3.6) [‡]	0.30 [§] (–0.46–1.05)	.44
Control	20.6 (7.5)	0.34 (2.8)	0.54 (3.2)		
SPPB					
Active [¶]	7.5 (1.9)	0.50 (1.26)	0.86 (1.38)**	0.11 [§] (–0.21–0.42)	.51
Control ^{††}	7.5 (2.0)	0.51 (1.21)	0.77 (1.45)**		
Chair-stand time, s ^{‡‡}					
Active ^{§§}	17.1 (15.2, 21.2)	–1.4 (–3.3–0.4)	–2.5 (–4.2 to –0.6)**	–1.01 [§] (–1.77 to –0.19)	.018
Control	17.6 (14.6, 20.6)	–1.0 (–3.0–1.1)	–1.2 (–3.3–0.8)**		
Balance test ^{¶¶}					
Active ^{¶¶}	3.0 (2.0, 4.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	N.A.	.89
Control ^{††}	3.0 (2.0, 4.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)		
Gait speed, m/s					
Active [¶]	0.8 (0.2)	0.03 (0.11)	0.07 (0.12)**	0.01 [§] (–0.02–0.04)	.46
Control ^{***}	0.8 (0.2)	0.03 (0.10)	0.05 (0.12)**		

N.A., not applicable; SPPB, Short Physical Performance Battery.

*The *P* value represents the time × treatment interaction derived from a mixed model (MMRM) adjusting for age, sex, and baseline protein intake.†Baseline: *n* = 179, week 7: *n* = 155, week 13: *n* = 139.‡*P* value < .01 derived from MMRM assessing the within-group change from baseline (time effect).§MMRM: active: *n* = 144, control: 158.||Baseline: *n* = 192, week 7: *n* = 169, week 13: *n* = 154.¶Baseline: *n* = 184, week 7: *n* = 159, week 13: *n* = 143.***P* value < .001 derived from MMRM assessing the within-group change from baseline (time effect).††Baseline: *n* = 196, week 7: *n* = 173, week 13: *n* = 158.

‡‡Median and interquartile range (IQR) presented since data had non-normal distributions. Data were log-transformed to enable a MMRM analysis.

§§Baseline: *n* = 162, week 7: *n* = 141, week 13: *n* = 126.|||Baseline: *n* = 170, week 7: *n* = 152, week 13: *n* = 138.¶¶Median and IQR presented because data were categorical, *P* value derived from Mann-Whitney test for nonparametric means. The within-group effect of time was not assessed because the MMRM could not be performed on these categorical data.***Baseline: *n* = 196, week 7: *n* = 172, week 13: *n* = 158.

In the other primary outcome measurement, SPPB, we also did not observe an intervention effect. This is likely explained by the unexpected positive and significant time effect both in the intervention and control groups. Furthermore, the SPPB is by nature a categorical score, and is less sensitive to changes than a continuous numerical scale. Although the significant changes in the chair-stand test did not result in significant differences in the overall SPPB score, the

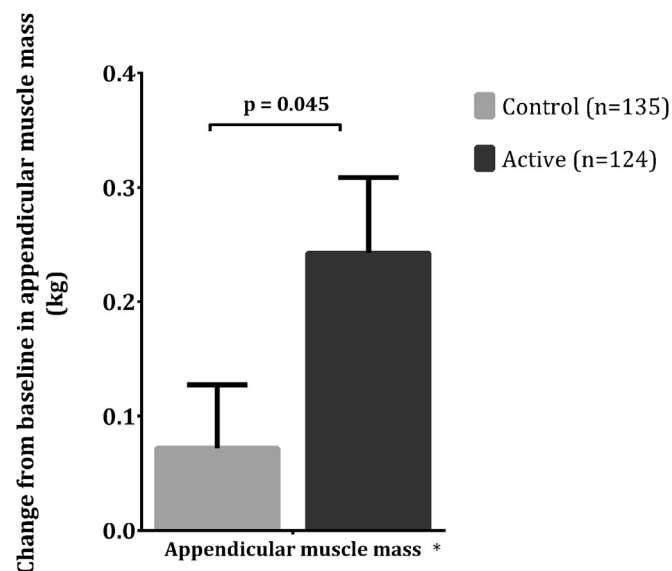
improvement we observed could be clinically meaningful. The chair-stand test is a robust measure of lower-extremity function because it requires lower-body strength, power, and good balance and coordination.⁵⁰ Poor chair-stand performance is an independent risk factor for physical disability, hospitalization, and mortality.⁵¹

As such, the sarcopenic screening measures of handgrip strength and SPPB may not be appropriate outcomes for measuring effects of sarcopenia interventions. We urge future researchers to carefully select sensitive and specific outcomes for sarcopenia, such as lower-extremity strength and function.

Although this study was performed among a robust sample of independently living older adults with mobility limitations, we could not include the full spectrum of older adults in the population at large. Groups such as those recovering from hospitalization and immobilization might benefit from nutritional supplementation, even while potentially unable to exercise. Although structured physical activity programs are not always practical or feasible, and maintaining good compliance is often problematic,⁵² ideally, this nutrition intervention would be combined with exercise. A recent study demonstrated a reduction in major mobility impairment after a long-term structured physical activity program.³⁶ These interventions, taken together, address 2 major mediating and reversible factors of sarcopenia and physical frailty, and have the potential to prolong mobility, independence, and quality of life.

Conclusion

We present here a 13-week intervention of a vitamin D and leucine-enriched whey protein oral nutritional supplement that resulted in improvements in muscle mass and lower-extremity function among sarcopenic older adults. This study shows proof-of-principle that specific nutritional supplementation alone might benefit geriatric patients, especially relevant for those who are unable to

**Fig. 2.** Change (kg) in appendicular muscle mass from baseline to week 13 follow-up.

*The raw mean change from baseline to week 13 and SE. The *P* value represents the time × treatment interaction derived from a mixed model (MMRM) adjusting for age, sex, and baseline protein intake.

Table 3
Nutritional and Biochemical Outcomes

	Median (IQR)	Change From Baseline Median (IQR)	Estimated Between-Group Difference Mean (95% CI) Active – Control	P*
	Baseline	13 weeks		
Serum 25-hydroxyvitamin D (nmol/L) [†]				
Active [‡]	48.0 (34.0–66.0)	25.0 (14.0–39.0) [§]	34.2 (29.2–39.6) [¶]	<.001
Control	49.0 (34.0–65.0)	–6.0 (–11.0–0.00) [§]		
Serum IGF-1 (μg/L) [‡]				
Active ^{**}	113.0 (80.0–145.0)	9.0 (–2.0–23.0) [§]	12.6 (7.4–18.1) [¶]	<.001
Control	114.0 (90.0–139.0)	–1.5 (–12.0–14.0)		
Non-supplementary dietary energy intake (kcal/day) ^{†,††}				
Active ^{‡‡}	1698 (1423–2028)	–124 (–395–161) ^{§§}	31.7 (–52.6–122.5) ^{¶¶}	.41
Control	1612 (1407–1918)	–127 (–372–160) ^{§§}		
Non-supplementary dietary protein intake (g/kg BW/day) ^{†,††}				
Active ^{‡‡}	1.0 (0.9–1.2)	–0.1 (–0.2–0.1)	0.02 (–0.05–0.09) ^{¶¶}	.56
Control	1.0 (0.8–1.2)	–0.1 (–0.2–0.1) ^{§§}		
Total dietary energy intake including supplement (kcal/day) ^{††,¶¶}				
Active ^{‡‡}	1698 (1423–2028)	166 (–95–458)	N.A.	.92
Control	1612 (1407–1918)	165 (–122–463)		
Total dietary protein intake including supplement (g/kg BW/day) ^{††,¶¶}				
Active ^{‡‡}	1.0 (0.9–1.2)	0.5 (0.3–0.6)	N.A.	<.001
Control	1.0 (0.8–1.2)	–0.1 (–0.2–0.1)		

N.A., not applicable.

*The P value represents the time × treatment interaction derived from a mixed model (MMRM) adjusting for age, sex, and baseline protein intake.

[†]Median and IQR presented since data had non-normal distributions. Data were log-transformed to enable a MMRM analysis.

[‡]Baseline: n = 180, week 13: n = 143.

[§]P value <.001 derived from MMRM assessing the within-group change from baseline (time effect).

^{||}Baseline: n = 191, week 13: n = 157.

[¶]MMRM: active: n = 144, control: 158.

^{**}Baseline: n = 182, week 13: n = 143.

^{††}Data calculated based on 3-day dietary intake records, including 1 week day and 1 weekend day on the week of baseline and 13-week follow-up. Energy and nutrient contributions by the supplement were estimated on an individual level by average reported compliance completed during the week of dietary assessment. This proportion was multiplied by the nutrient composition of both the active and control supplements and added to the total habitual intakes.

^{‡‡}Baseline: n = 176, week 13: n = 138.

^{§§}P value <.05 derived from MMRM assessing the within-group change from baseline (time effect).

^{||||}Baseline: n = 188, week 13: n = 152.

^{¶¶}Median and IQR presented because data had non-normal distributions. P value derived from Mann-Whitney test for nonparametric means. Within-group effect of time was not assessed because the MMRM was not performed on these data.

exercise. These results warrant further investigations into the role of a specific nutritional supplement as part of a multimodal approach to prevent adverse outcomes among older adults at risk for disability.

Acknowledgments

We are grateful to the participants for the time and energy they spent while taking part in this trial. We are thankful to the energy and commitment of all research staff members at each study site. We are thankful to Peter Zuithoff and Rene Eijkemans, PhD, from the Julius Center, Utrecht University Medical Center, Utrecht, the Netherlands, for acting as independent statisticians and repeating the analyses presented here. Thank you to Yves Boirie, MD, PhD, University of Clermont-Ferrand, France; Kit Roes, PhD, Utrecht University, The Netherlands; and Renger Witkamp, PhD, Wageningen University, the Netherlands, for their role as the data monitoring committee of this study.

Supplementary Data

Supplementary Data related to this article can be found online at <http://dx.doi.org/10.1016/j.jamda.2015.05.021>.

References

- Morley JE, Vellas B, van Kan GA, et al. Frailty consensus: A call to action. *J Am Med Dir Assoc* 2013;14:392–397.
- Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010;39:412–423.
- Narici MV, Maffulli N. Sarcopenia: Characteristics, mechanisms and functional significance. *Br Med Bull* 2010;95:139–159.
- Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: Evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001;56:M146–M156.
- Cederholm T, Cruz-Jentoft AJ, Maggi S. Sarcopenia and fragility fractures. *Eur J Phys Rehabil Med* 2013;49:111–117.
- Guralnik JM, Simonsick EM, Ferrucci L, et al. A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;49:M85–M94.
- Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med* 1995;332:556–561.
- Kalyani RR, Corriere M, Ferrucci L. Age-related and disease-related muscle loss: The effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol* 2014;2:819–829.
- Morley JE, Argiles JM, Evans WJ, et al. Nutritional recommendations for the management of sarcopenia. *J Am Med Dir Assoc* 2010;11:391–396.
- Houston DK, Nicklas BJ, Ding J, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: The Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr* 2008;87:150–155.
- Scott D, Blizzard L, Fell J, et al. Associations between dietary nutrient intake and muscle mass and strength in community-dwelling older adults: The Tasmanian Older Adult Cohort Study. *J Am Geriatr Soc* 2010;58:2129–2134.
- Wicherts IS, van Schoor NM, Boeke AJ, et al. Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab* 2007;92:2058–2065.
- Beasley JM, Wertheim BC, LaCroix AZ, et al. Biomarker-calibrated protein intake and physical function in the Women's Health Initiative. *J Am Geriatr Soc* 2013;61:1863–1871.
- Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, et al. Effect of vitamin D on falls: A meta-analysis. *JAMA* 2004;291:1999–2006.
- Boirie Y. Fighting sarcopenia in older frail subjects: Protein fuel for strength, exercise for mass. *J Am Med Dir Assoc* 2013;14:140–143.
- Forbes SC, Little JP, Candow DG. Exercise and nutritional interventions for improving aging muscle health. *Endocrine* 2012;42:29–38.
- Bauer J, Biolo G, Cederholm T, et al. Evidence-based recommendations for optimal dietary protein intake in older people: A position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc* 2013;14:542–559.

18. Breen L, Phillips SM. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing. *Nutr Metab (Lond)* 2011;8:68.
19. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 2005;19:422–424.
20. Deutz NE, Bauer JM, Barazzoni R, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. *Clin Nutr* 2014;33:929–936.
21. Chale A, Cloutier GJ, Hau C, et al. Efficacy of whey protein supplementation on resistance exercise-induced changes in lean mass, muscle strength, and physical function in mobility-limited older adults. *J Gerontol A Biol Sci Med Sci* 2013;68:682–690.
22. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, et al. Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* 2004;286:E321–E328.
23. Kramer IF, Poeze M, Luiking YC, et al. Basal and post-prandial muscle protein synthesis rates are not reduced in sarcopenic elderly [abstract]. *Clin Nutr* 2014; 33:S127.
24. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889–896.
25. Schuit AJ, Schouten EG, Westerterp KR, Saris WH. Validity of the Physical Activity Scale for the Elderly (PASE): According to energy expenditure assessed by the doubly labeled water method. *J Clin Epidemiol* 1997;50:541–546.
26. Mahoney FI, Barthel DW. Functional evaluation: The Barthel Index. *Md State Med J* 1965;14:61–65.
27. The EuroQol Group. Euro-Qol, a new facility for the measurement of health-related quality of life. *Health Policy* 1990;16:199–208.
28. Bunout B, Barrera G, de la Maza P, et al. Effects of nutritional supplementation and resistance training on muscle strength in free living elders. Results of one year follow. *J Nutr Health Aging* 2004;8:68–75.
29. Scognamiglio R, Piccolotto R, Negut C, et al. Oral amino acids in elderly subjects: Effect on myocardial function and walking capacity. *Gerontology* 2005; 51:302–308.
30. Rejeski WJ, Marsh AP, Chmelo E, et al. The lifestyle interventions and Independence for elders Pilot (LIFE-P): 2-year follow-up. *J Gerontol A Biol Sci Med Sci* 2009;64:462–467.
31. Allison PD. *Handling Missing Data by Maximum Likelihood*. Orlando, FL: SAS Global Forum; 2012.
32. Kramer IF, Poeze M, Luiking YC, et al. Basal and post-prandial muscle protein synthesis rates are not reduced in sarcopenic elderly [abstract]. Geneva, Switzerland: 36th ESPEN Congress; 2014.
33. Luiking YC, Deutz NE, Memelink RG, et al. Postprandial muscle protein synthesis is higher after a high whey protein, leucine-enriched supplement than after a dairy-like product in healthy older people: A randomized controlled trial. *Nutr J* 2014;13:9.
34. Life Study Investigators, Pahor M, Blair SN, Espeland M, et al. Effects of a physical activity intervention on measures of physical performance: Results of the lifestyle interventions and independence for Elders Pilot (LIFE-P) study. *J Gerontol A Biol Sci Med Sci* 2006;61:1157–1165.
35. Latham NK, Harris BA, Bean JF, et al. Effect of a home-based exercise program on functional recovery following rehabilitation after hip fracture: A randomized clinical trial. *JAMA* 2014;311:700–708.
36. Pahor M, Guralnik JM, Ambrosius WT, et al. Effect of structured physical activity on prevention of major mobility disability in older adults: The LIFE study randomized clinical trial. *JAMA* 2014;311:2387–2396.
37. Cruz-Jentoft AJ, Landi F, Schneider SM, et al. Prevalence of and interventions for sarcopenia in ageing adults: A systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age Ageing* 2014;43:748–759.
38. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 1994; 330:1769–1775.
39. Tieland M, van de Rest O, Dirks ML, et al. Protein supplementation improves physical performance in frail elderly people: A randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* 2012;13:720–726.
40. Lauretani F, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: An operational diagnosis of sarcopenia. *J Appl Physiol* (1985) 2003;95:1851–1860.
41. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol* (1985) 2000;89:81–88.
42. Gallagher D, Visser M, De Meersman RE, et al. Appendicular skeletal muscle mass: Effects of age, gender, and ethnicity. *J Appl Physiol* (1985) 1997;83:229–239.
43. Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and interpretation and uses of Dietary Reference Intakes, et al. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: The National Academies Press; 2015.
44. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* 2006;291:E381–E387.
45. Adamo ML, Farrar RP. Resistance training, and IGF involvement in the maintenance of muscle mass during the aging process. *Ageing Res Rev* 2006;5: 310–331.
46. Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv Exp Med Biol* 2014;810:500–525.
47. Salles J, Chanet A, Giraudet C, et al. 1,25(OH)₂-vitamin D₃ enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. *Mol Nutr Food Res* 2013;57:2137–2146.
48. Abizanda P, Navarro JL, Garcia-Tomas MI, et al. Validity and usefulness of hand-held dynamometry for measuring muscle strength in community-dwelling older persons. *Arch Gerontol Geriatr* 2012;54:21–27.
49. Tieland M, Verdijk LB, de Groot LC, van Loon LJ. Handgrip strength does not represent an appropriate measure to evaluate changes in muscle strength during an exercise intervention program in frail older people. *Int J Sport Nutr Exerc Metab* 2015;25:27–36.
50. Hardy R, Cooper R, Shah I, et al. Is chair rise performance a useful measure of leg power? *Ageing Clin Exp Res* 2010;22:412–418.
51. Cesari M, Kritchevsky SB, Newman AB, et al. Added value of physical performance measures in predicting adverse health-related events: Results from the Health, Aging and Body Composition Study. *J Am Geriatr Soc* 2009;57:251–259.
52. Zech A, Drey M, Freiburger E, et al. Residual effects of muscle strength and muscle power training and detraining on physical function in community-dwelling prefrail older adults: A randomized controlled trial. *BMC Geriatr* 2012;12:68.